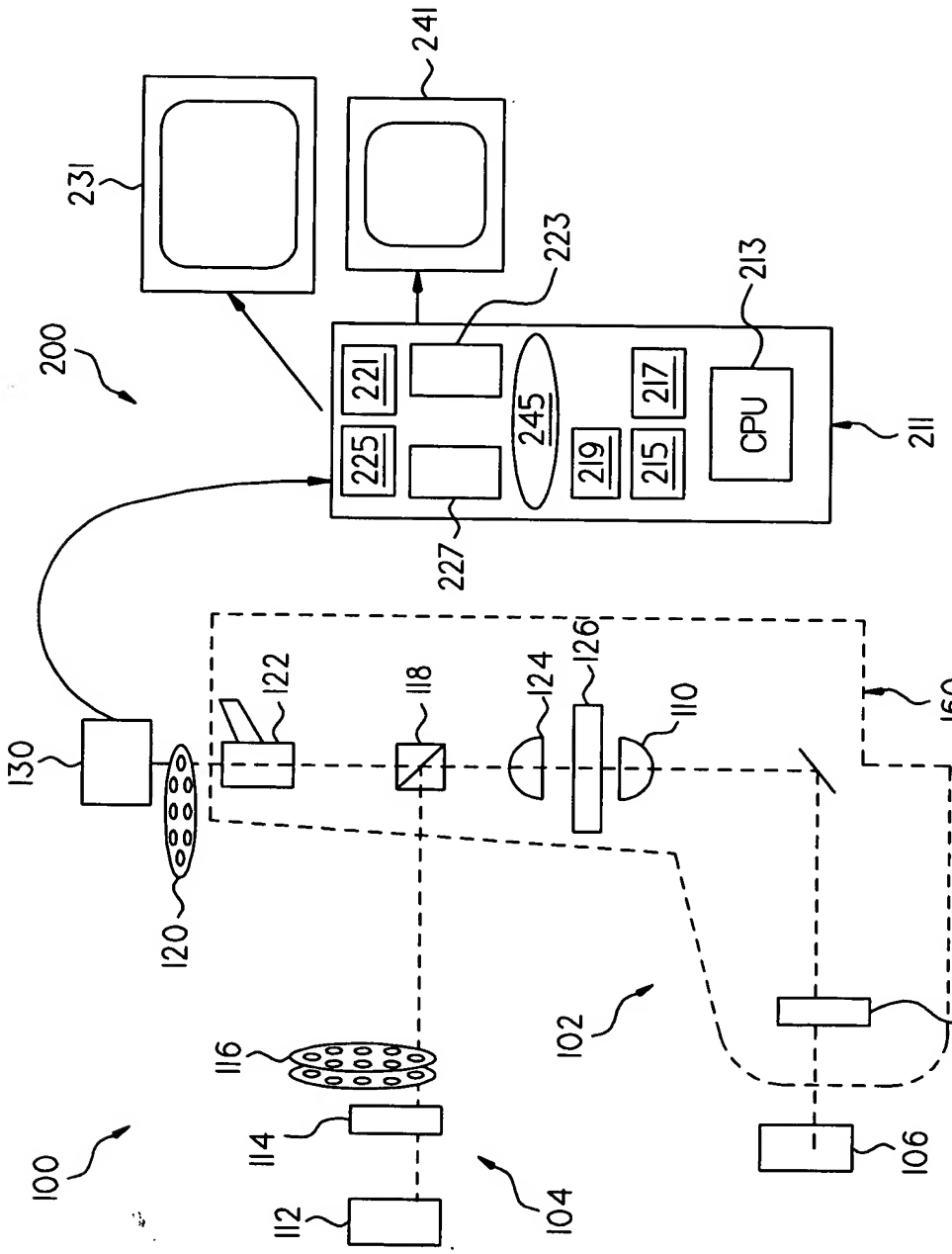
241
231
223
213
200
221
225
245
219
217
215
CPU
211
227
122
118
124
126
110
160
130
120
116
102
108
114
100
104
106
112

Fig-1

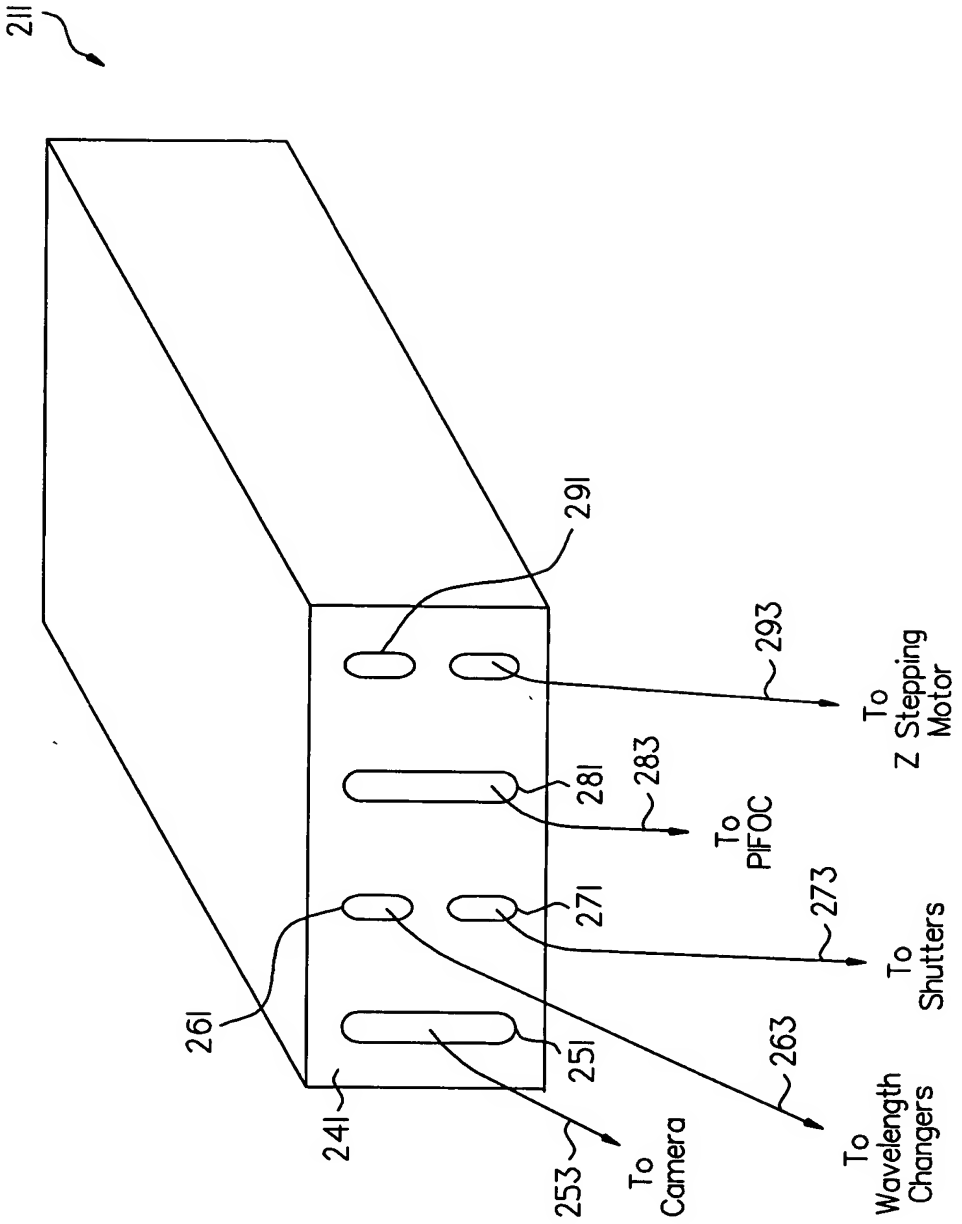
211
291
293
To
Z Stepping
Motor
281
283
To
PIFOC
271
273
To
Shutters
261
251
263
241
253
To
Camera
To
Wavelength
Changers

Fig. 2

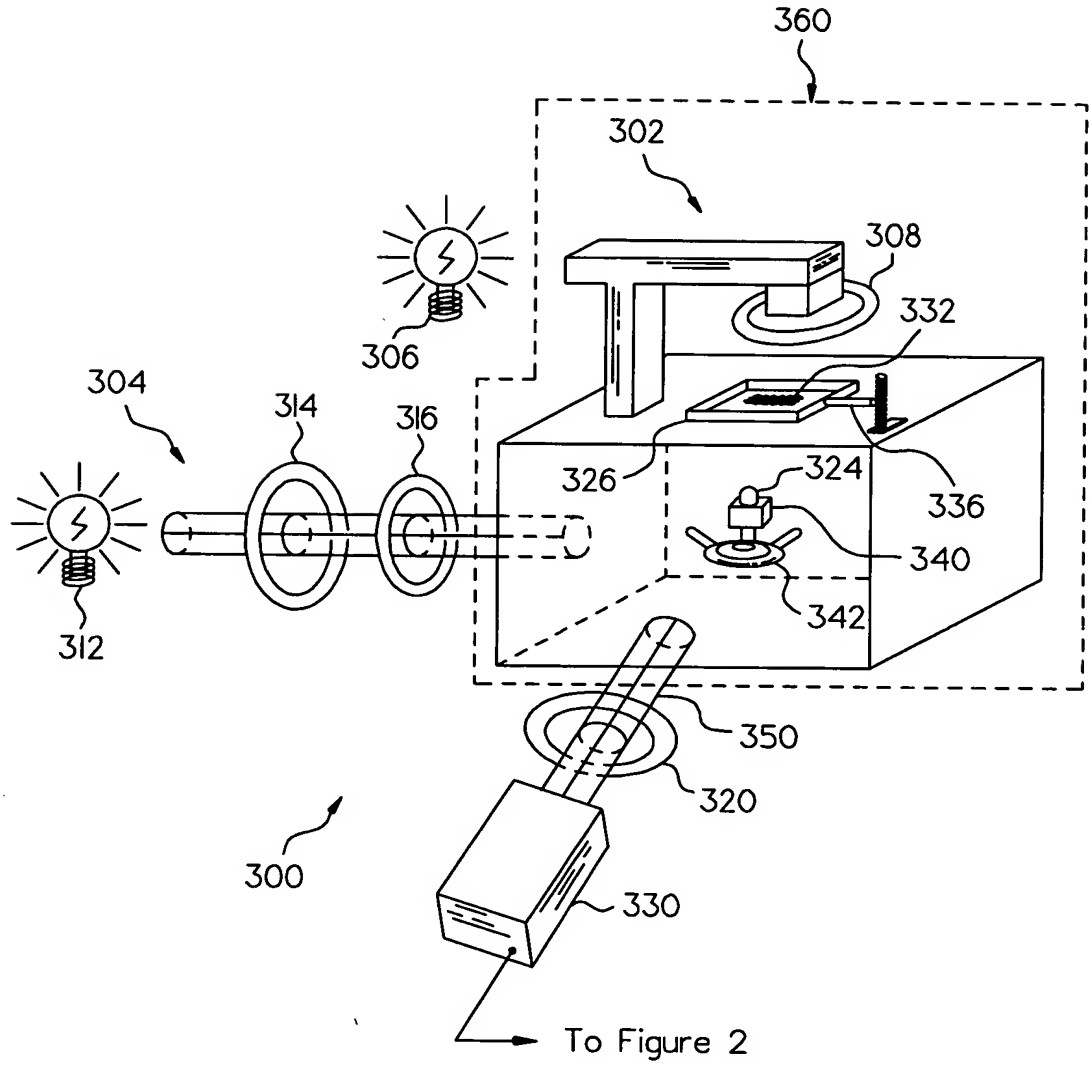
360
302
308
332
306
304
314
316
326
324
336
340
342
312
350
320
300
330
To Figure 2

Fig. 3

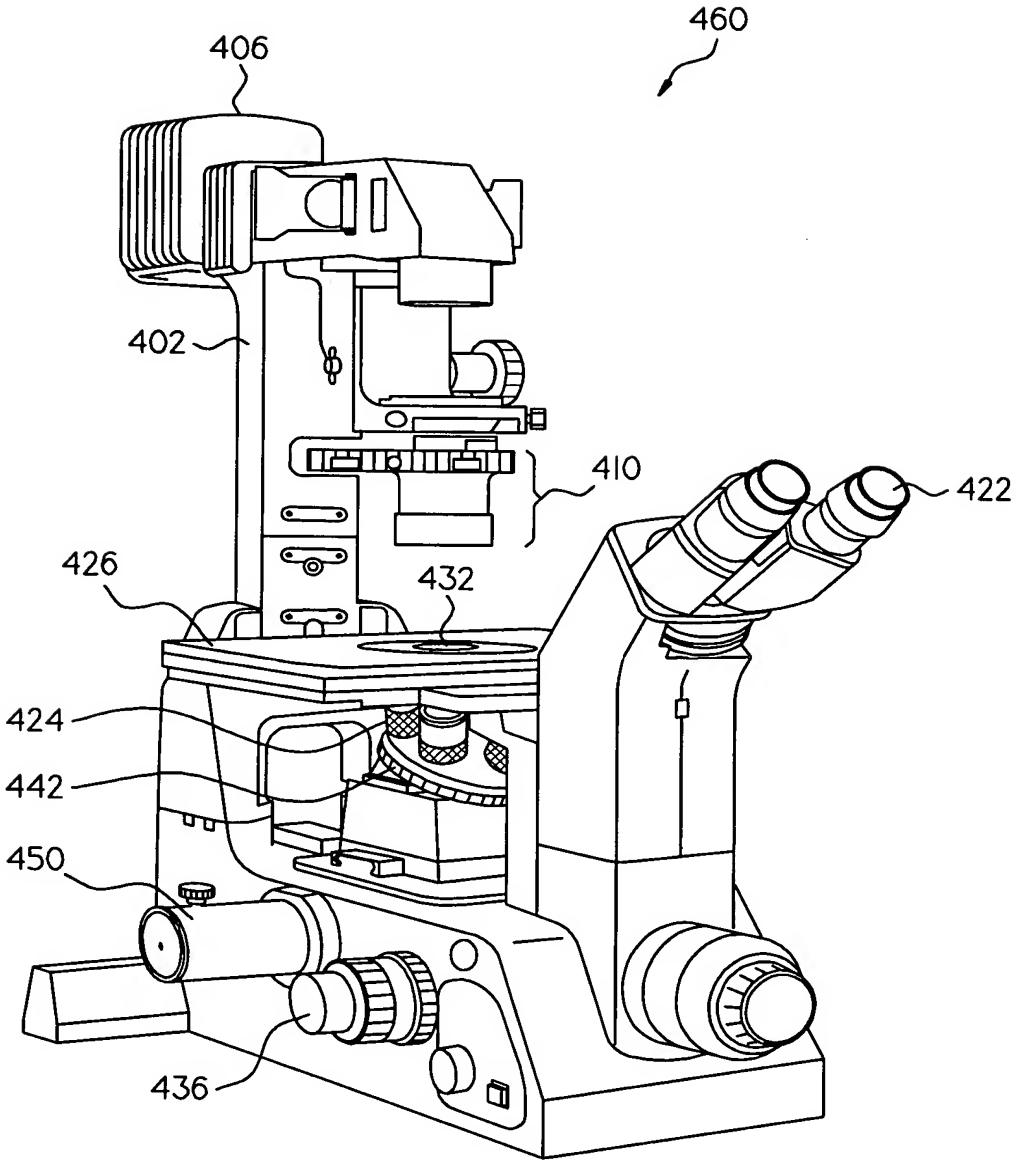

Fig. 4

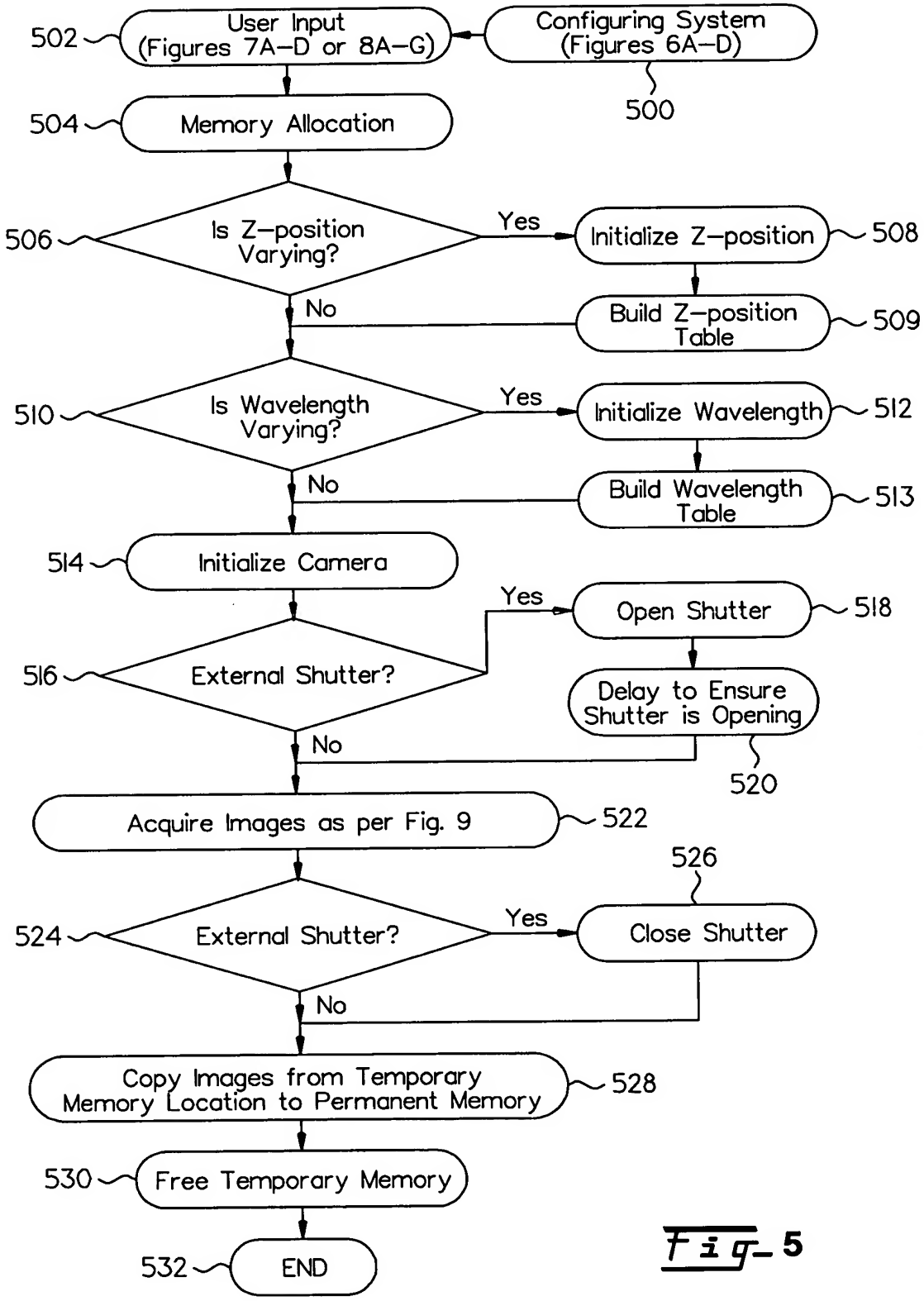
User Input (Figures 7A–D or 8A–G)
502
Configuring System (Figures 6A–D)
500
Memory Allocation
504
Is Z–position Varying?
506
Yes
Initialize Z–position
508
No
Build Z–position Table
509
Is Wavelength Varying?
510
Yes
Initialize Wavelength
512
No
Build Wavelength Table
513
Initialize Camera
514
External Shutter?
516
Yes
Open Shutter
518
Delay to Ensure Shutter is Opening
520
No
Acquire Images as per Fig. 9
522
External Shutter?
524
526
Yes
Close Shutter
No
Copy Images from Temporary Memory Location to Permanent Memory
528
Free Temporary Memory
530
END
532

Fig. 5

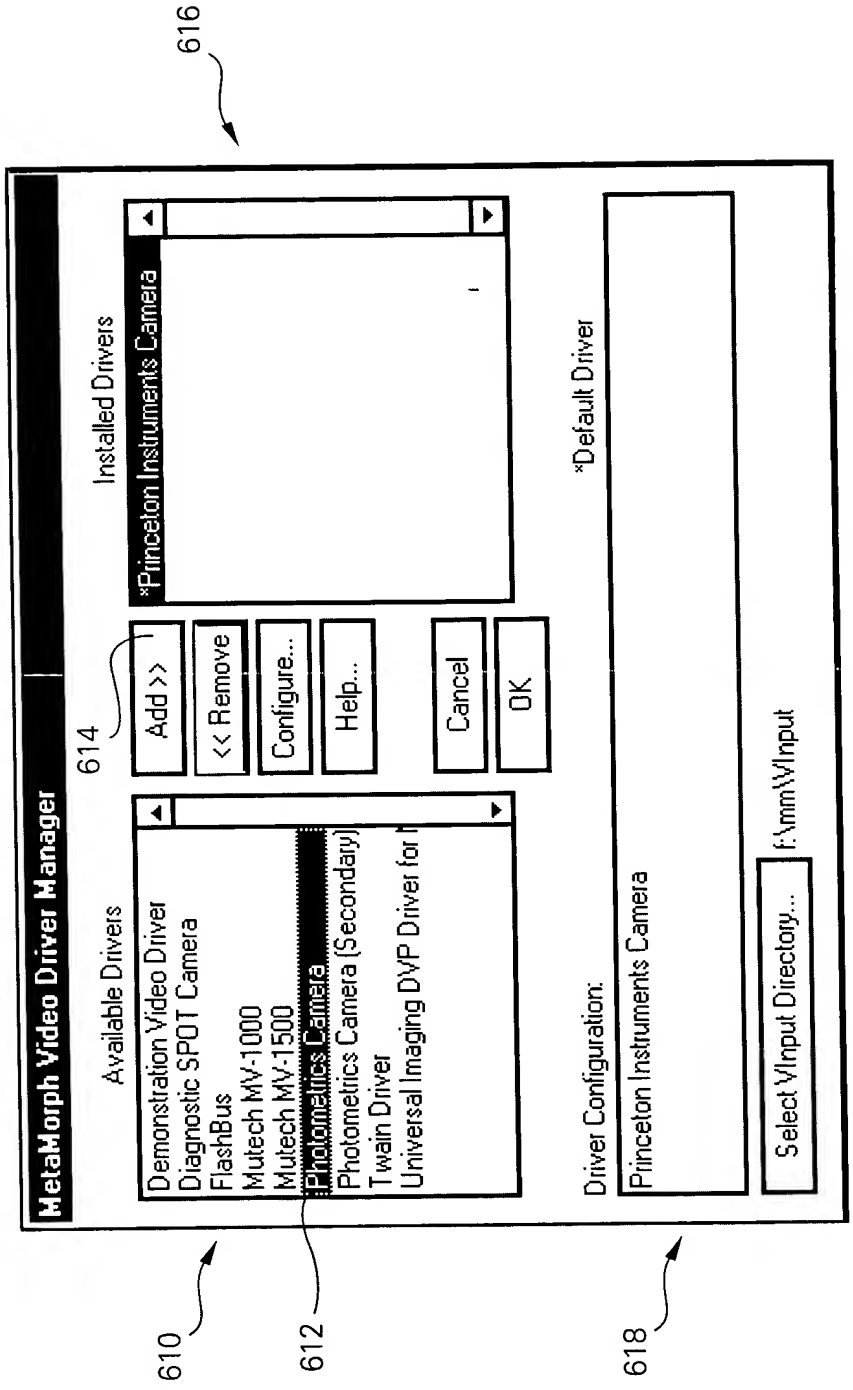
616
MetaMorph Video Driver Manager
Available Drivers
Demonstration Video Driver
Diagnostic SPOT Camera
FlashBus
Mutech MV-1000
Mutech MV-1500
Photometrics Camera
Photometrics Camera (Secondary)
Twain Driver
Universal Imaging DVP Driver for I
614
Add >>
<< Remove
Configure...
Help...
Cancel
OK
Installed Drivers
*Princeton Instruments Camera
*Default Driver
Driver Configuration:
Princeton Instruments Camera
Select VInput Directory...
f:\mmVInput
610
612
618

Fig. 6A

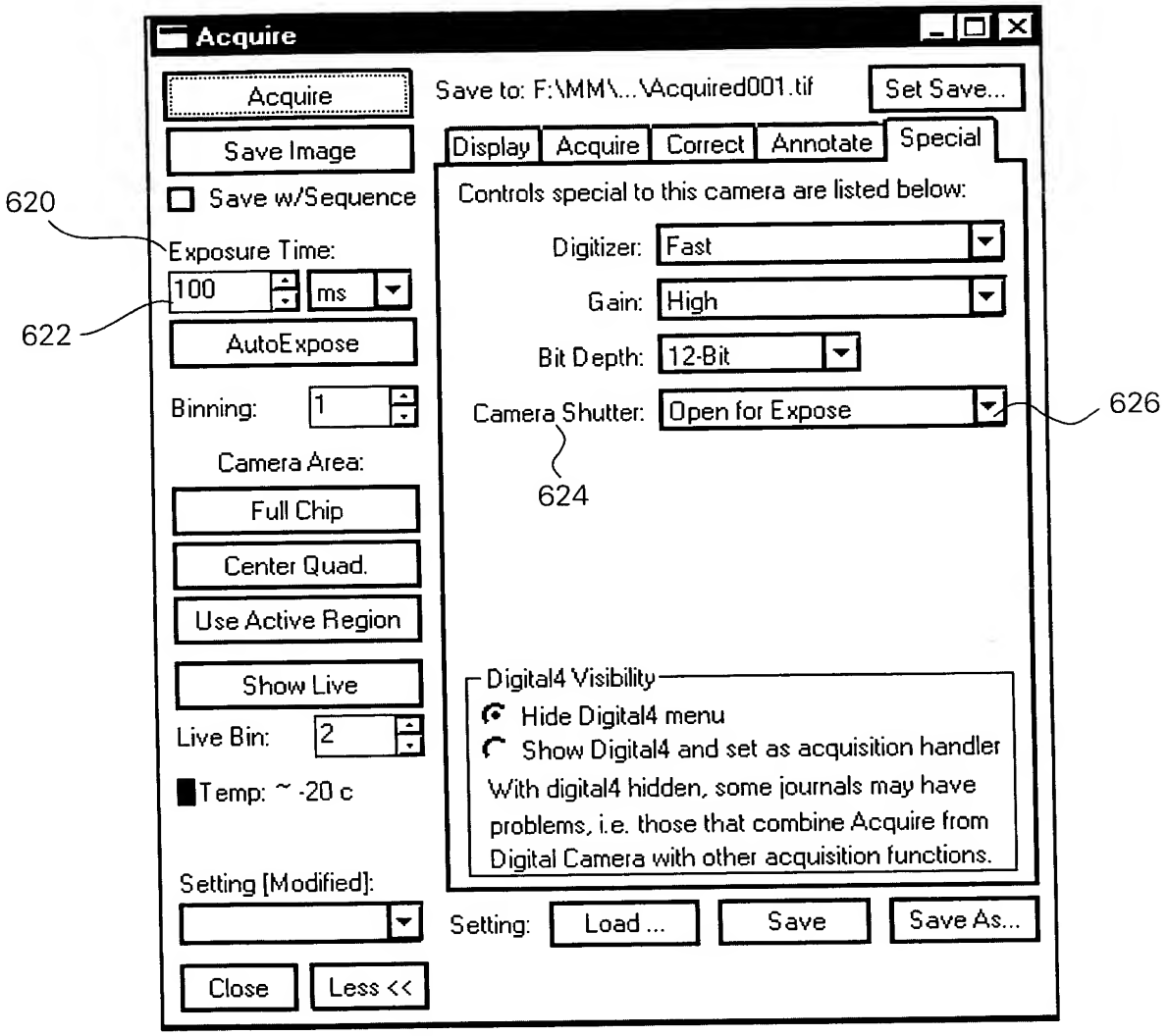
Acquire
Acquire
Save Image
Save w/Sequence
620
Exposure Time:
100
ms
622
AutoExpose
Binning:
1
Camera Area:
Full Chip
Center Quad.
Use Active Region
Show Live
Live Bin:
2
Temp: ~ -20 c
Setting [Modified]:
Close
Less <<
Save to: F:\MM\...\Acquired001.tif
Set Save...
Display
Acquire
Correct
Annotate
Special
Controls special to this camera are listed below:
Digitizer:
Fast
Gain:
High
Bit Depth:
12-Bit
Camera Shutter:
Open for Expose
626
624
Digital4 Visibility
Hide Digital4 menu
Show Digital4 and set as acquisition handler
With digital4 hidden, some journals may have problems, i.e. those that combine Acquire from Digital Camera with other acquisition functions.
Setting:
Load ...
Save
Save As...

Fig. 6B

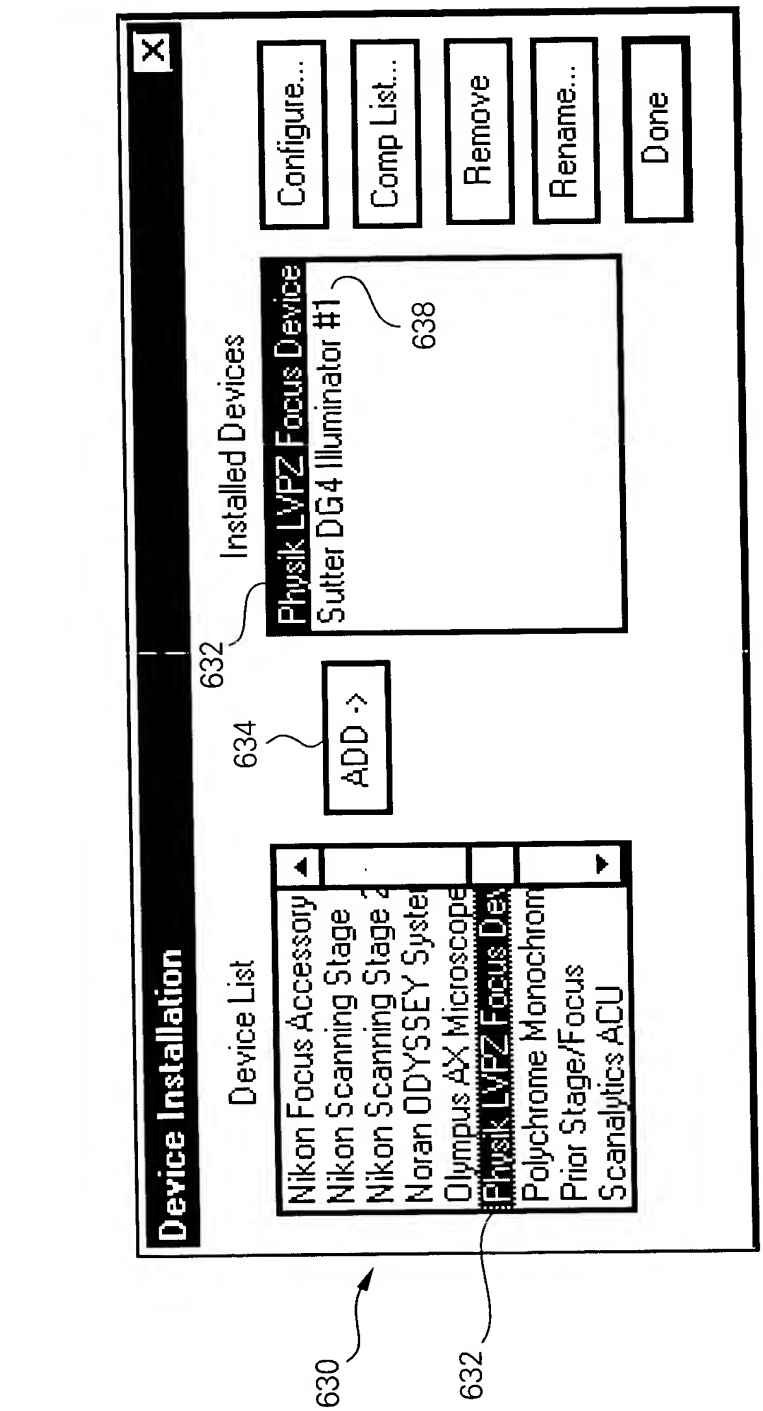
Device Installation
X
Device List
Nikon Focus Accessory
Nikon Scanning Stage
Nikon Scanning Stage 2
Noran ODYSSEY Syste
Olympus AX Microscope
Physik LVPZ Focus Dev
Polychrome Monochrom
Prior Stage/Focus
Scanalytics ACU
634
ADD ->
632
Installed Devices
Physik LVPZ Focus Device
Sutter DG4 Illuminator #1
638
Configure...
Comp List...
Remove
Rename...
Done
630
632

Fig- 6C

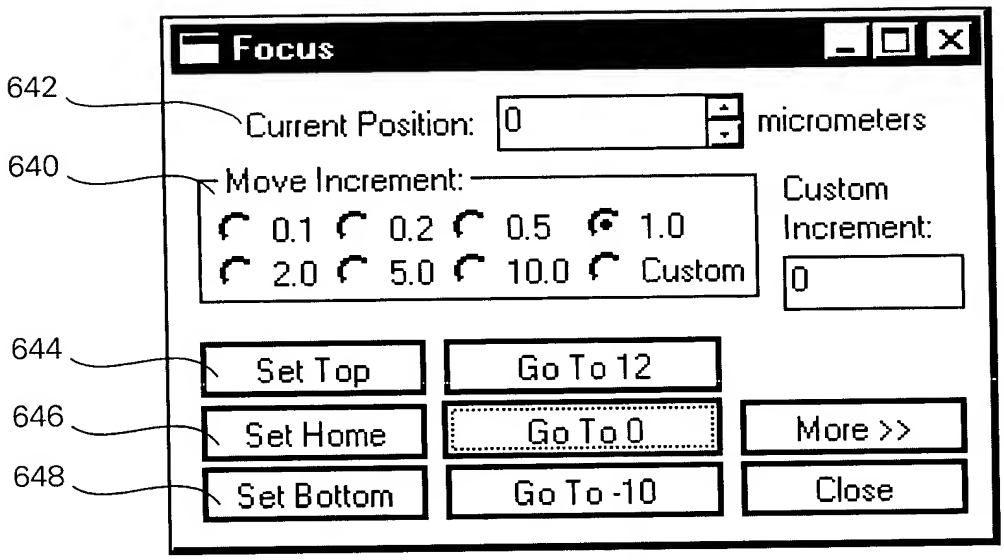
Focus
642
Current Position:
0
micrometers
640
Move Increment:
0.1
0.2
0.5
1.0
2.0
5.0
10.0
Custom
Custom Increment:
0
644
Set Top
Go To 12
646
Set Home
Go To 0
More >>
648
Set Bottom
Go To -10
Close

Fig. 6D

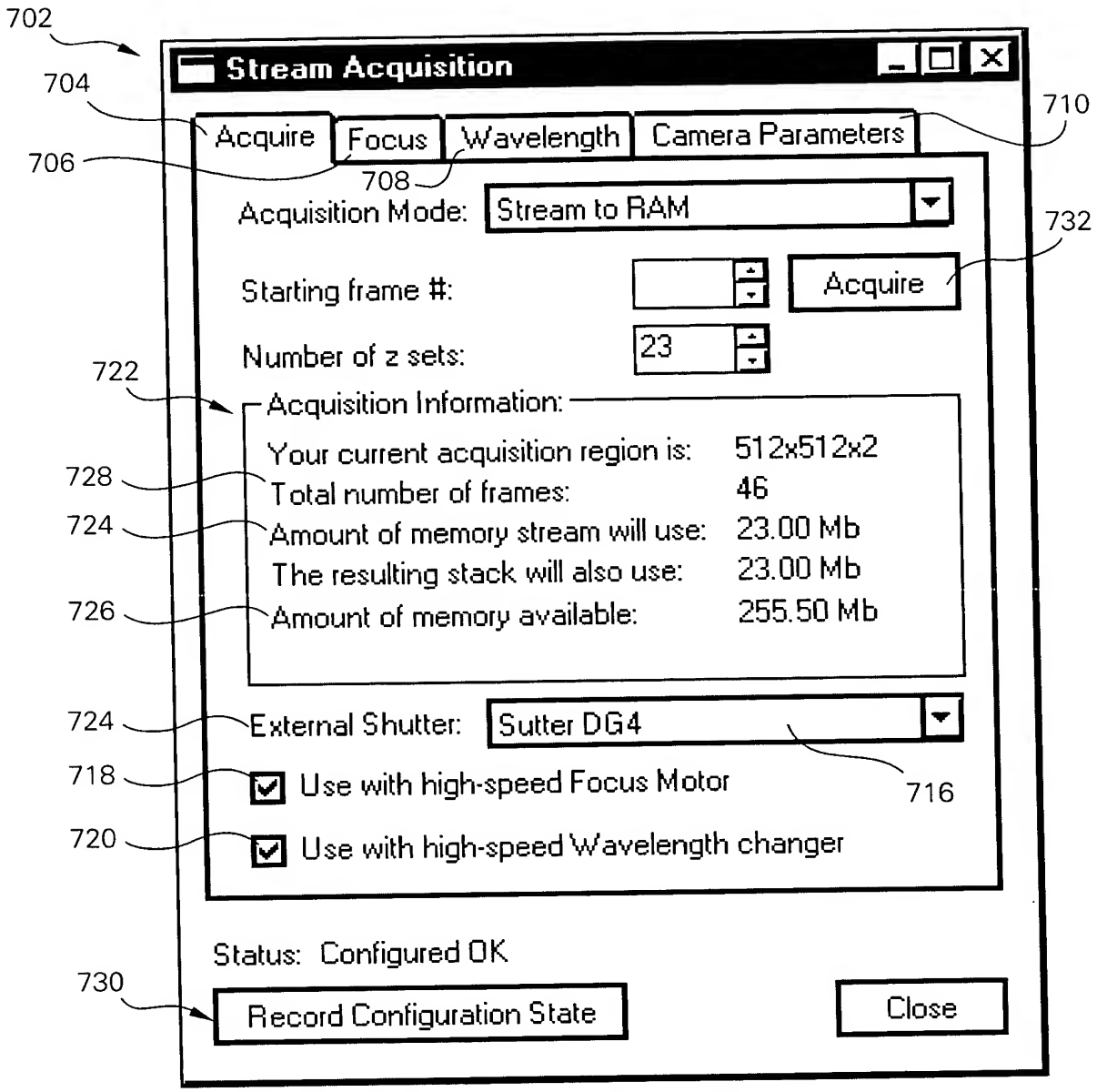
702
Stream Acquisition
704
710
Acquire
Focus
Wavelength
Camera Parameters
706
708
Acquisition Mode:
Stream to RAM
732
Starting frame #:
Acquire
Number of z sets:
23
722
Acquisition Information:
Your current acquisition region is:
512x512x2
728
Total number of frames:
46
724
Amount of memory stream will use:
23.00 Mb
The resulting stack will also use:
23.00 Mb
726
Amount of memory available:
255.50 Mb
724
External Shutter:
Sutter DG4
716
718
Use with high-speed Focus Motor
720
Use with high-speed Wavelength changer
Status: Configured OK
730
Record Configuration State
Close

Fig. 7A

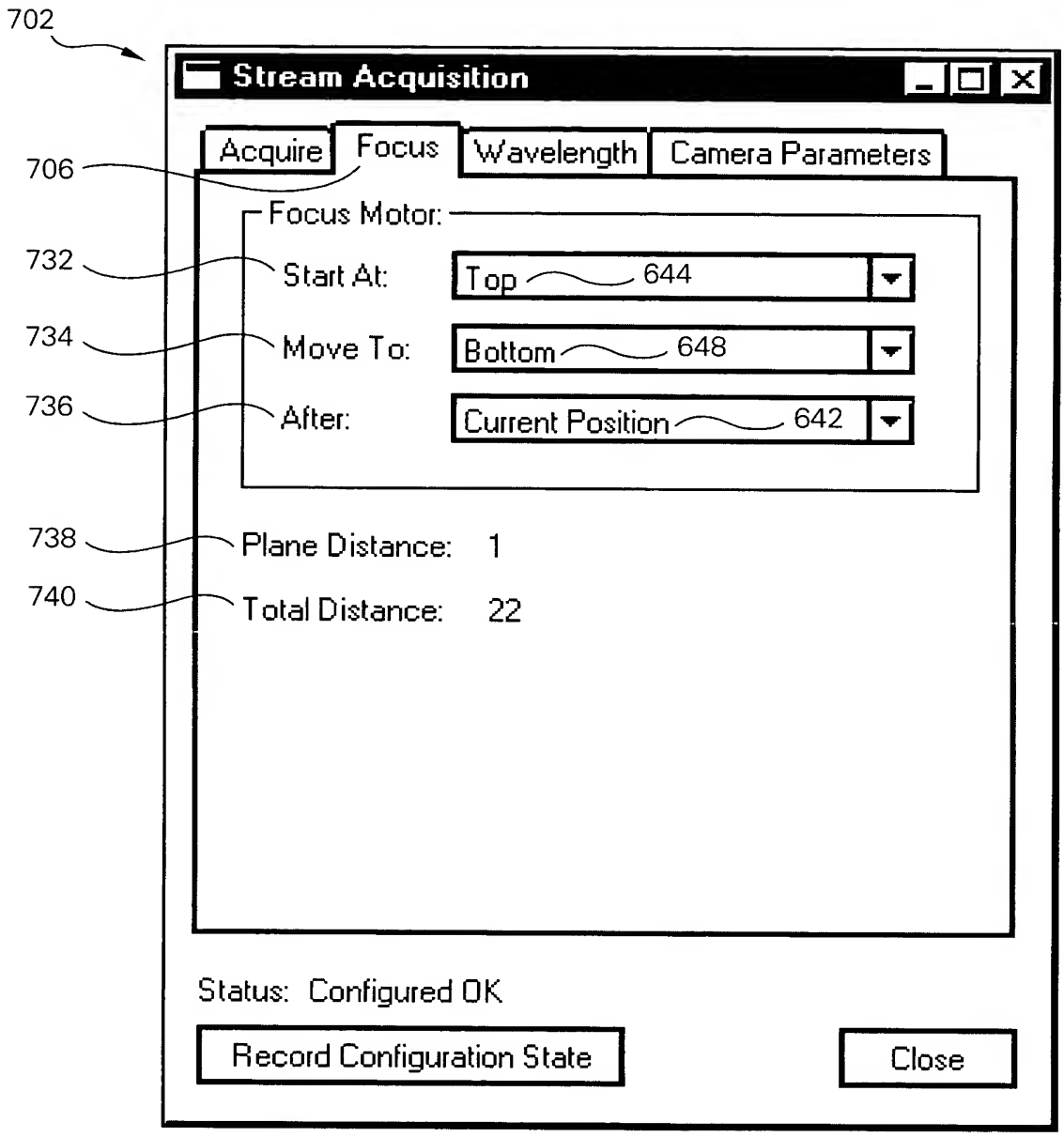
702
Stream Acquisition
Acquire
Focus
Wavelength
Camera Parameters
706
Focus Motor:
732
Start At:
Top
644
734
Move To:
Bottom
648
736
After:
Current Position
642
738
Plane Distance:
1
740
Total Distance:
22
Status: Configured OK
Record Configuration State
Close

Fig. 7B

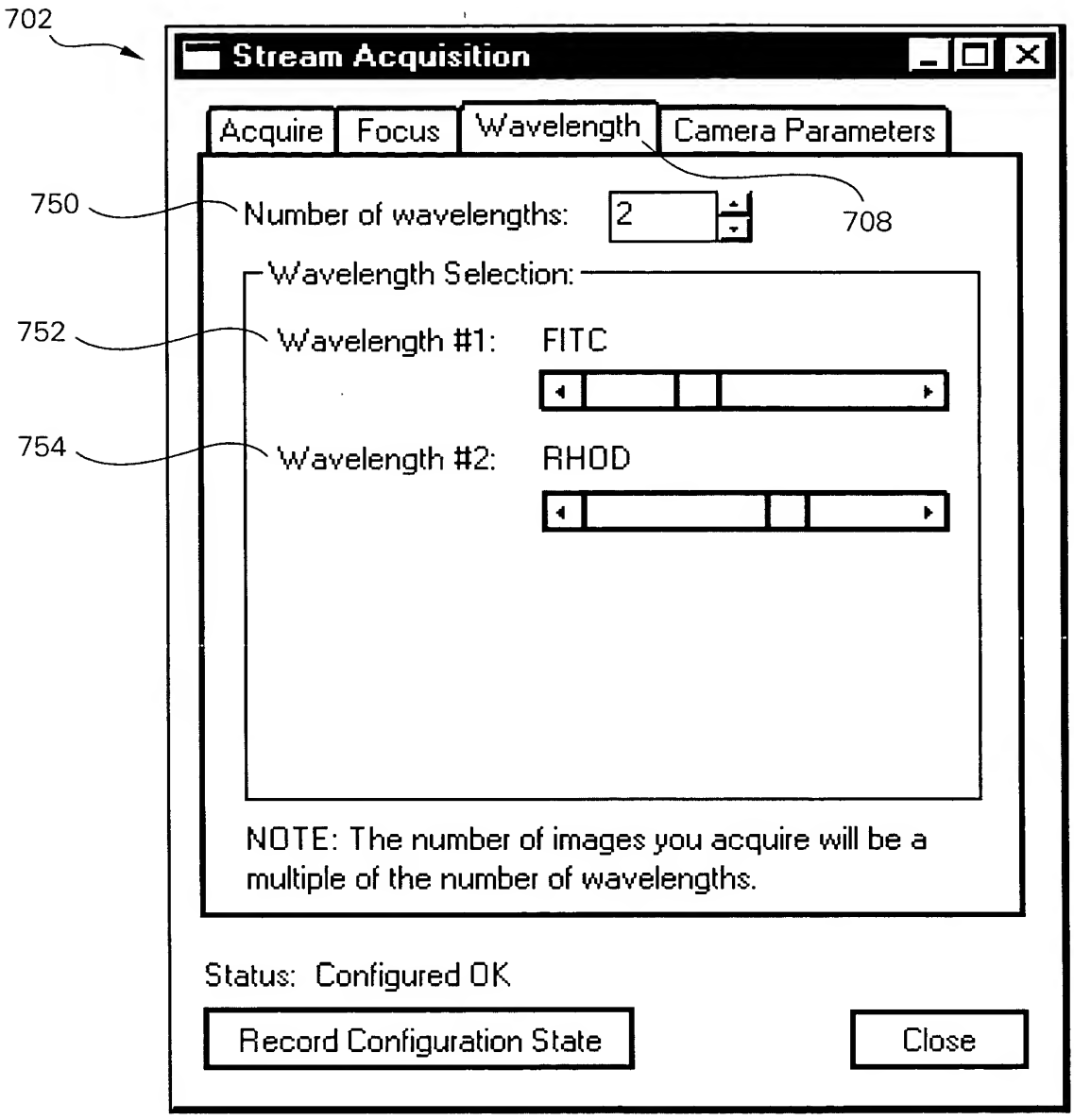
702
Stream Acquisition
Acquire
Focus
Wavelength
Camera Parameters
750
Number of wavelengths:
2
708
Wavelength Selection:
752
Wavelength #1:
FITC
754
Wavelength #2:
RHOD
NOTE: The number of images you acquire will be a multiple of the number of wavelengths.
Status: Configured OK
Record Configuration State
Close

Fig. 7C

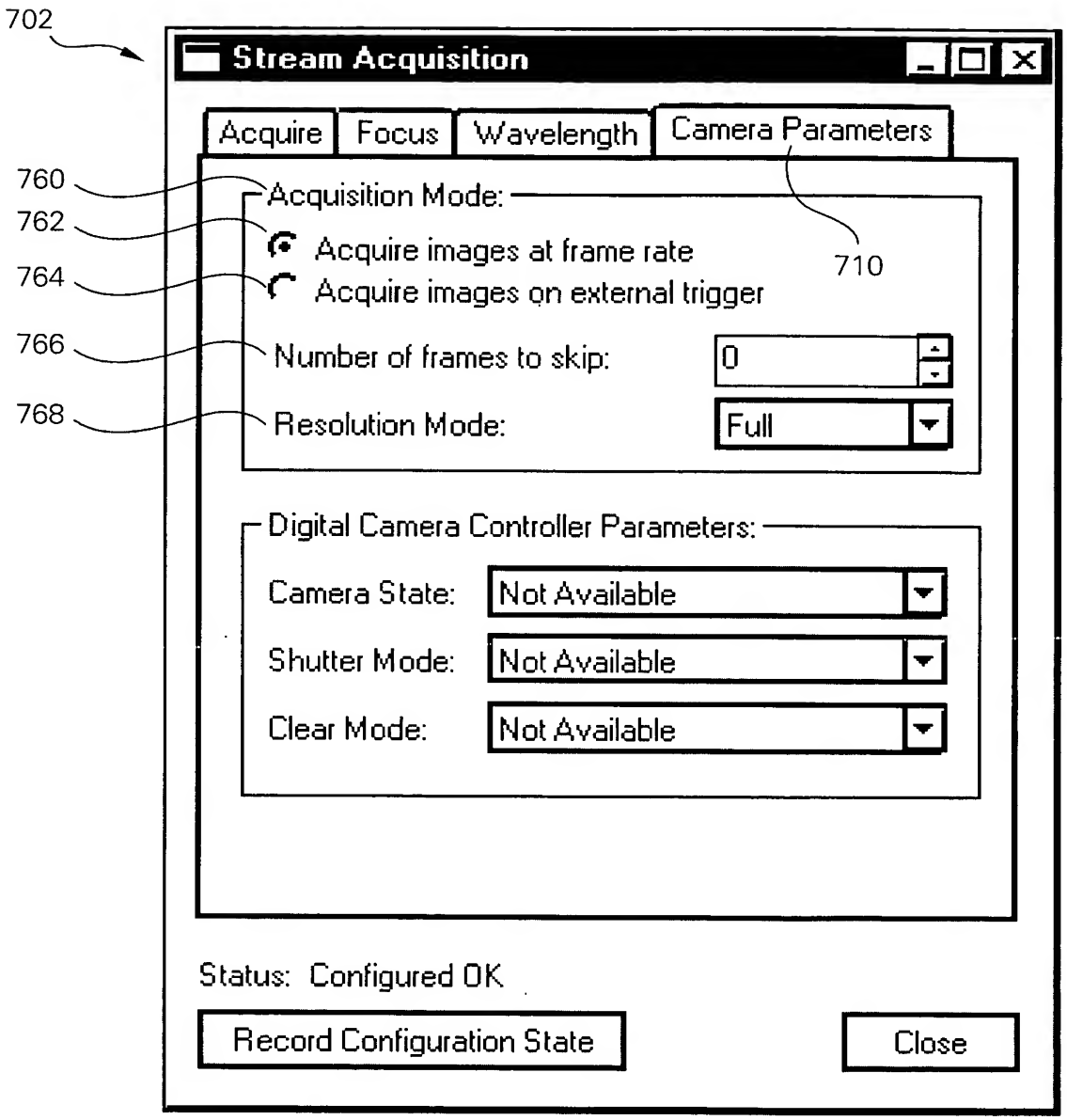
702
Stream Acquisition
Acquire
Focus
Wavelength
Camera Parameters
760
Acquisition Mode:
762
Acquire images at frame rate
764
Acquire images on external trigger
710
766
Number of frames to skip:
0
768
Resolution Mode:
Full
Digital Camera Controller Parameters:
Camera State:
Not Available
Shutter Mode:
Not Available
Clear Mode:
Not Available
Status: Configured OK
Record Configuration State
Close

Fig. 7D

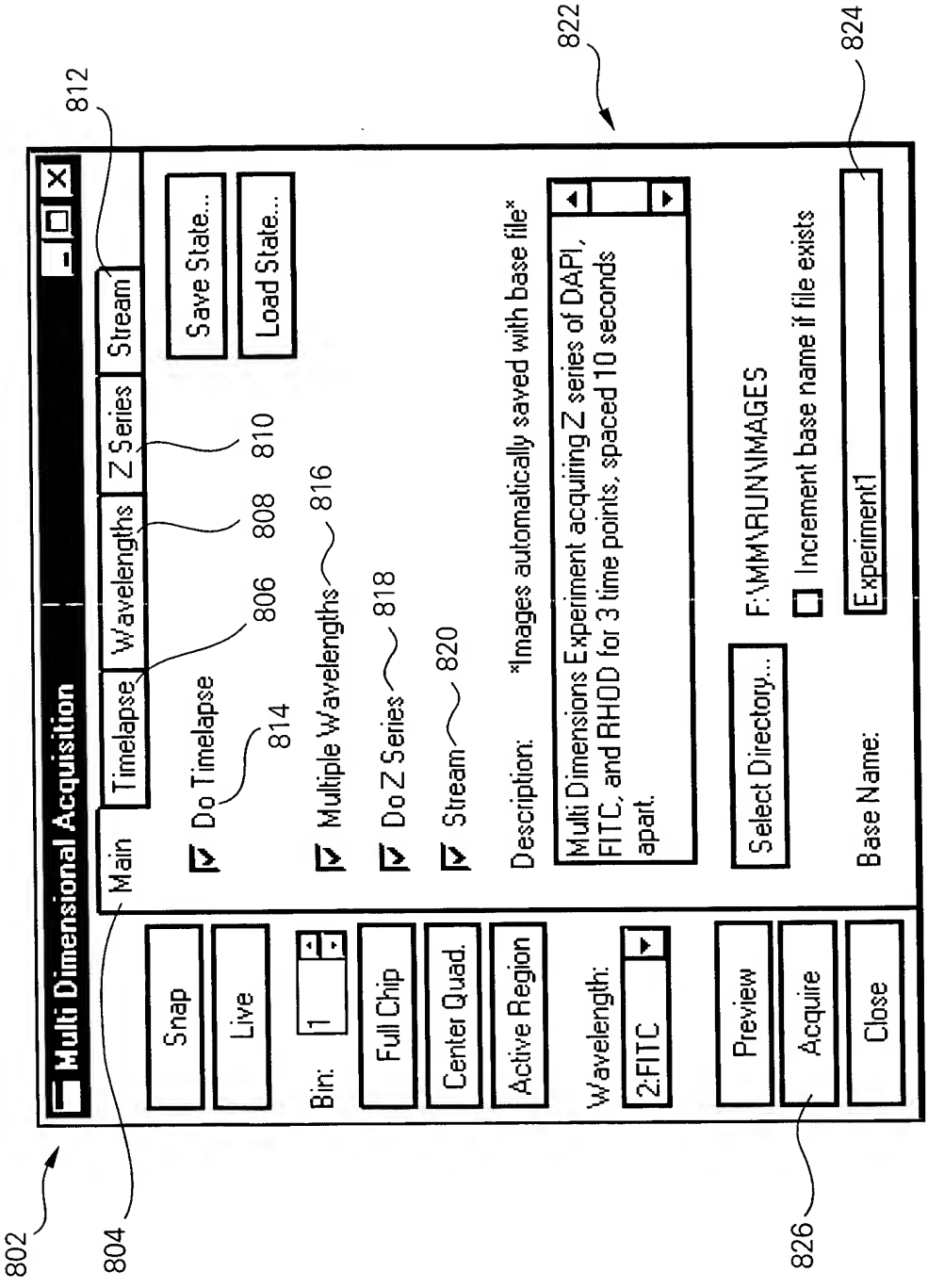
Multi Dimensional Acquisition
Main
Timelapse
Wavelengths
Z Series
Stream
Snap
Live
Bin:
1
Full Chip
Center Quad.
Active Region
Wavelength:
2:FITC
Preview
Acquire
Close
Do Timelapse
Multiple Wavelengths
Do Z Series
Stream
Save State...
Load State...
Description:
Images automatically saved with base file
Multi Dimensions Experiment acquiring Z series of DAPI, FITC, and RHOD for 3 time points, spaced 10 seconds apart.
Select Directory...
F:\MM\RUN\IMAGES
Increment base name if file exists
Base Name:
Experiment1
802
804
806
808
810
812
814
816
818
820
822
824
826

Fig. 8A

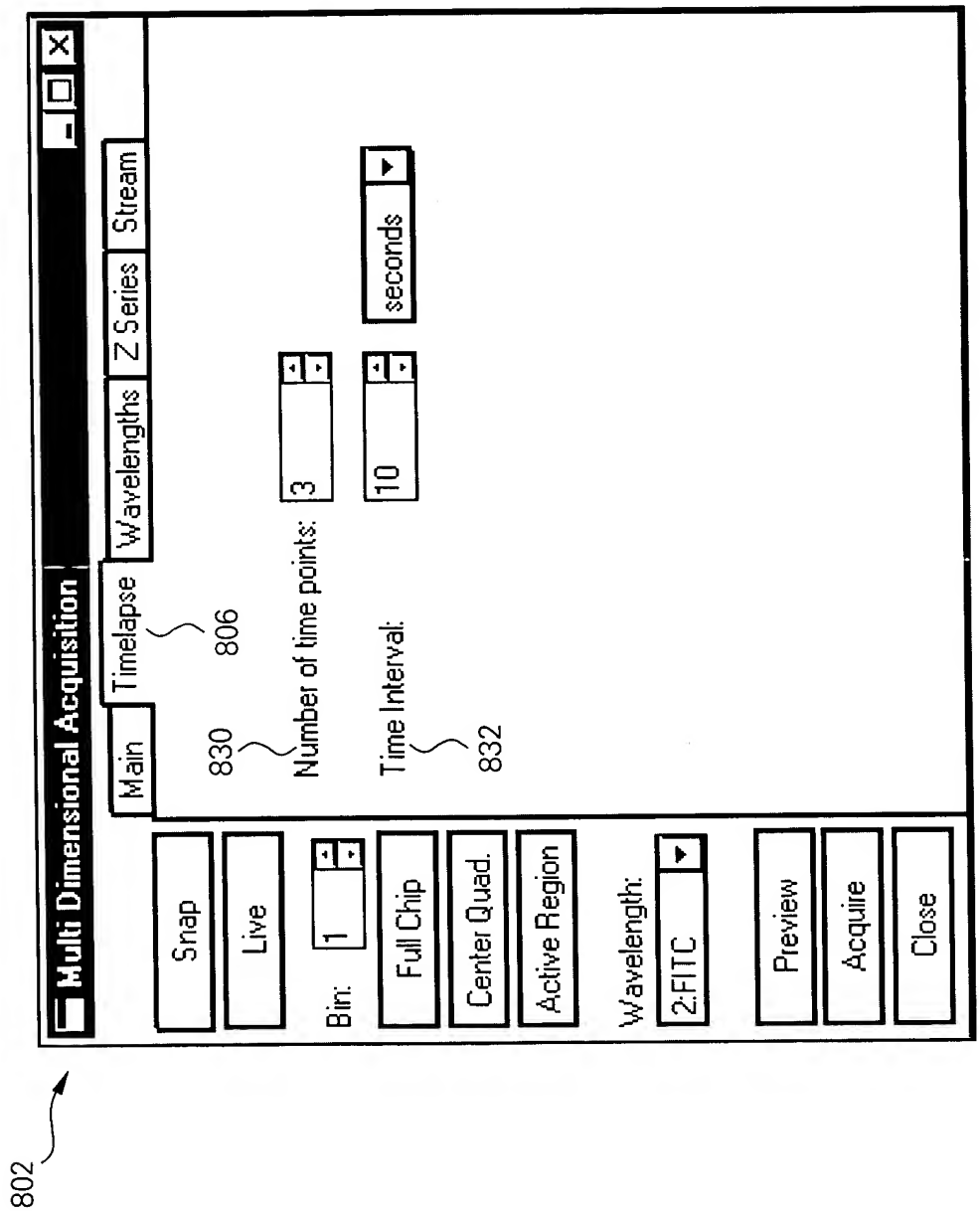
802
Multi Dimensional Acquisition
Main
Timelapse
Wavelengths
Z Series
Stream
806
Snap
Live
Bin:
1
Full Chip
Center Quad.
Active Region
Wavelength:
2:FITC
Preview
Acquire
Close
830
Number of time points:
3
Time Interval:
832
10
seconds

Fig. 8B

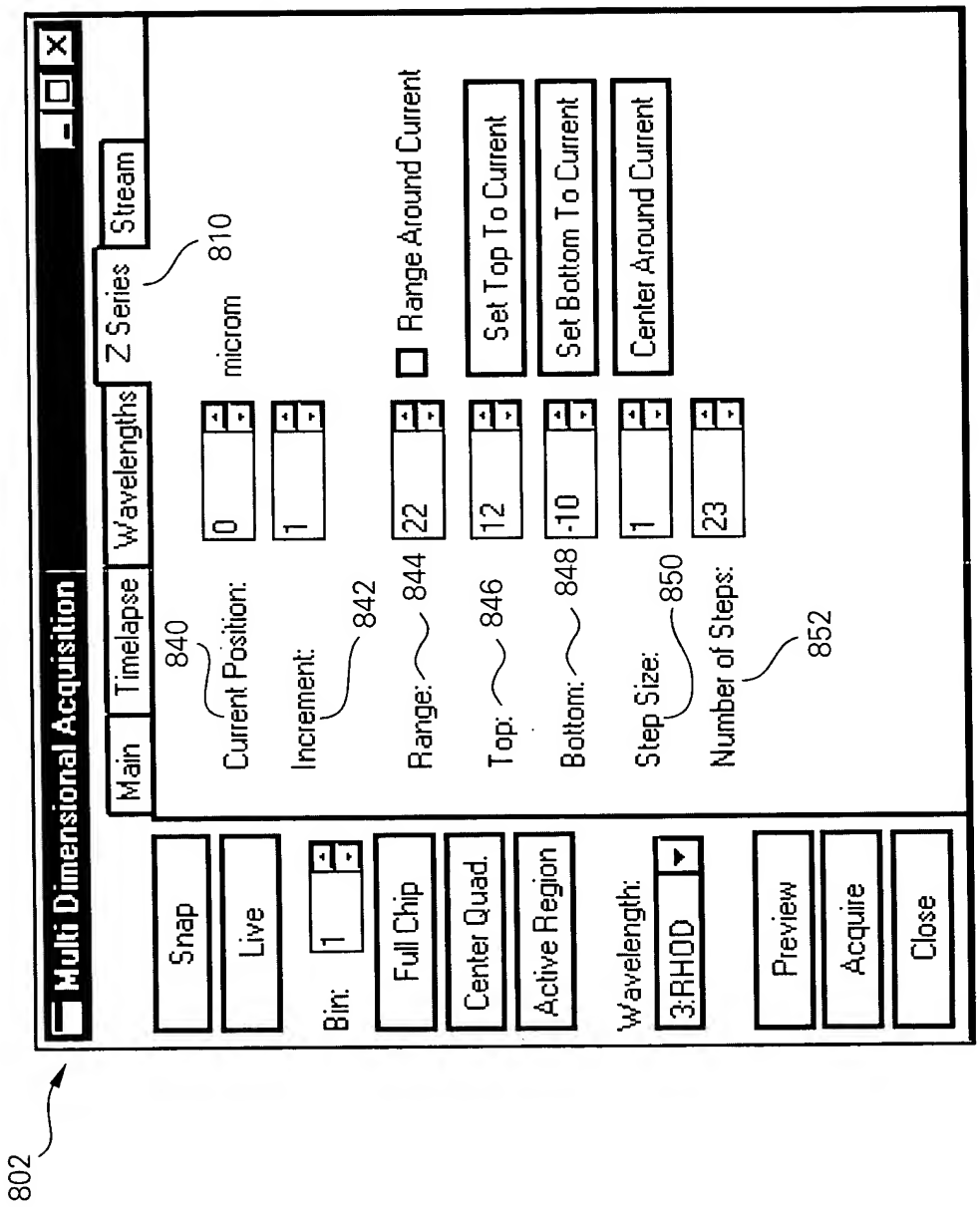
802
Multi Dimensional Acquisition
Main
Timelapse
Wavelengths
Z Series
Stream
Snap
Live
Bin:
1
Full Chip
Center Quad.
Active Region
Wavelength:
3:RHOD
Preview
Acquire
Close
840
Current Position:
0
microm
810
Increment:
1
842
Range:
844
22
Range Around Current
Top:
846
12
Set Top To Current
Bottom:
848
-10
Set Bottom To Current
Step Size:
850
1
Center Around Current
Number of Steps:
852
23

Fig. 8C

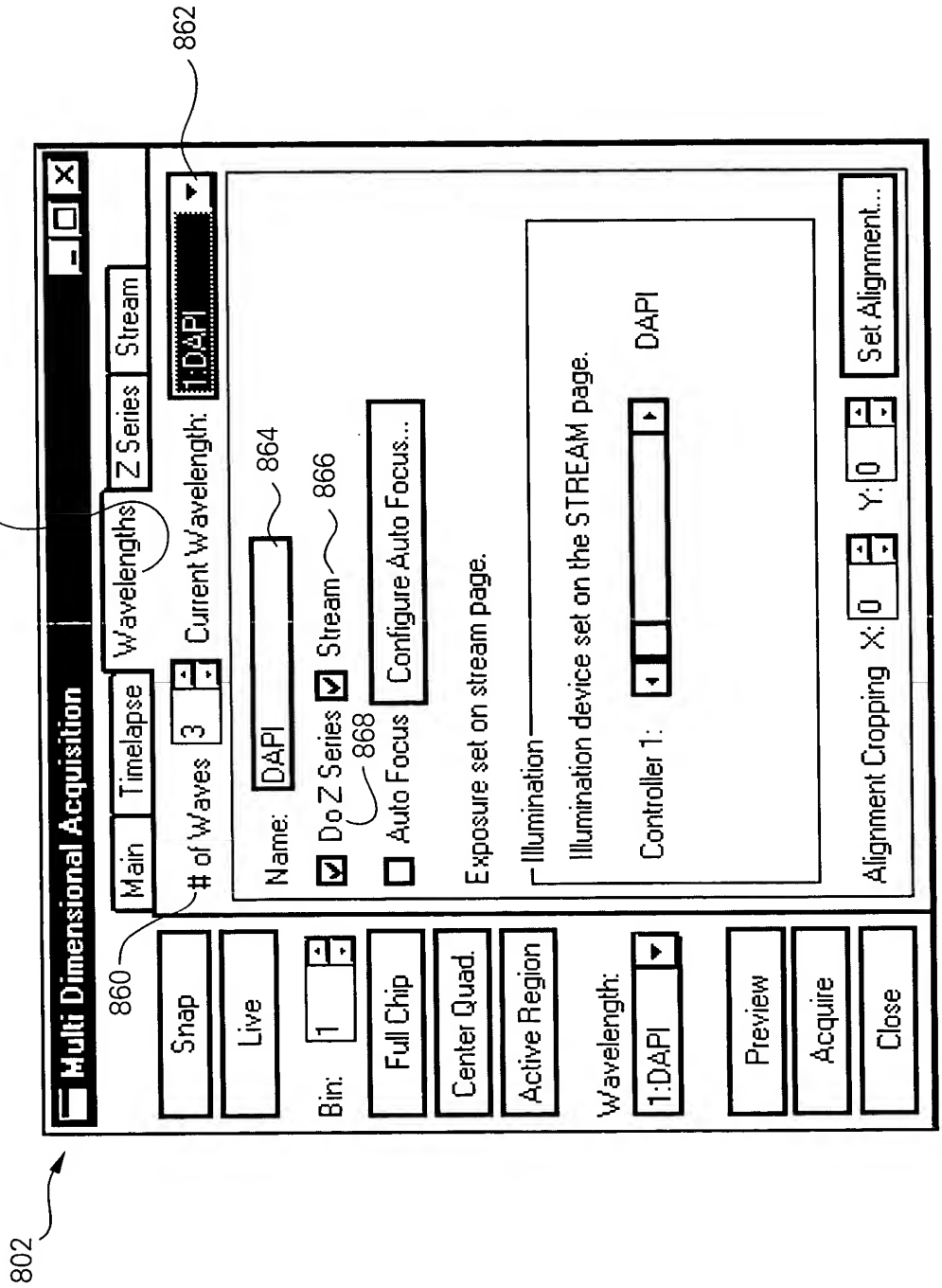
802
808
860
862
Multi Dimensional Acquisition
Snap
Live
Bin:
1
Full Chip
Center Quad.
Active Region
Wavelength:
1:DAPI
Preview
Acquire
Close
Main
Timelapse
Wavelengths
Z Series
Stream
# of Waves
3
Current Wavelength:
1:DAPI
Name:
DAPI
Do Z Series
Stream
Auto Focus
Configure Auto Focus...
Exposure set on stream page.
Illumination
Illumination device set on the STREAM page.
Controller 1:
DAPI
Alignment Cropping X:
0
Y:
0
Set Alignment...
864
866
868

Fig. 8D

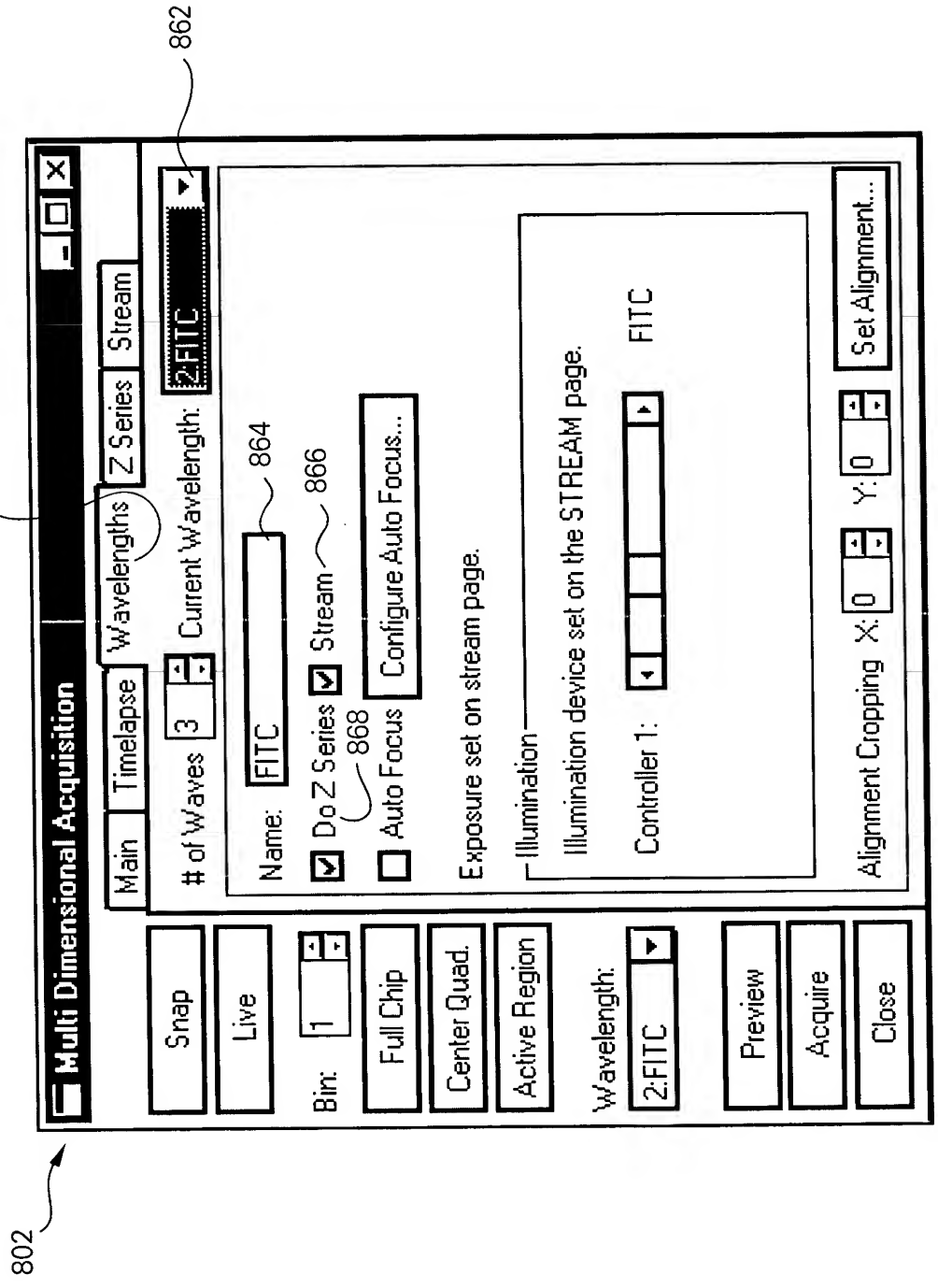
802
808
862
Multi Dimensional Acquisition
Main
Timelapse
Wavelengths
Z Series
Stream
Snap
Live
Bin:
1
Full Chip
Center Quad.
Active Region
Wavelength:
2:FITC
Preview
Acquire
Close
# of Waves
3
Current Wavelength:
2:FITC
Name:
FITC
864
Do Z Series
Stream
866
868
Auto Focus
Configure Auto Focus...
Exposure set on stream page.
Illumination
Illumination device set on the STREAM page.
Controller 1:
FITC
Alignment Cropping X:
0
Y:
0
Set Alignment...

Fig. 8E

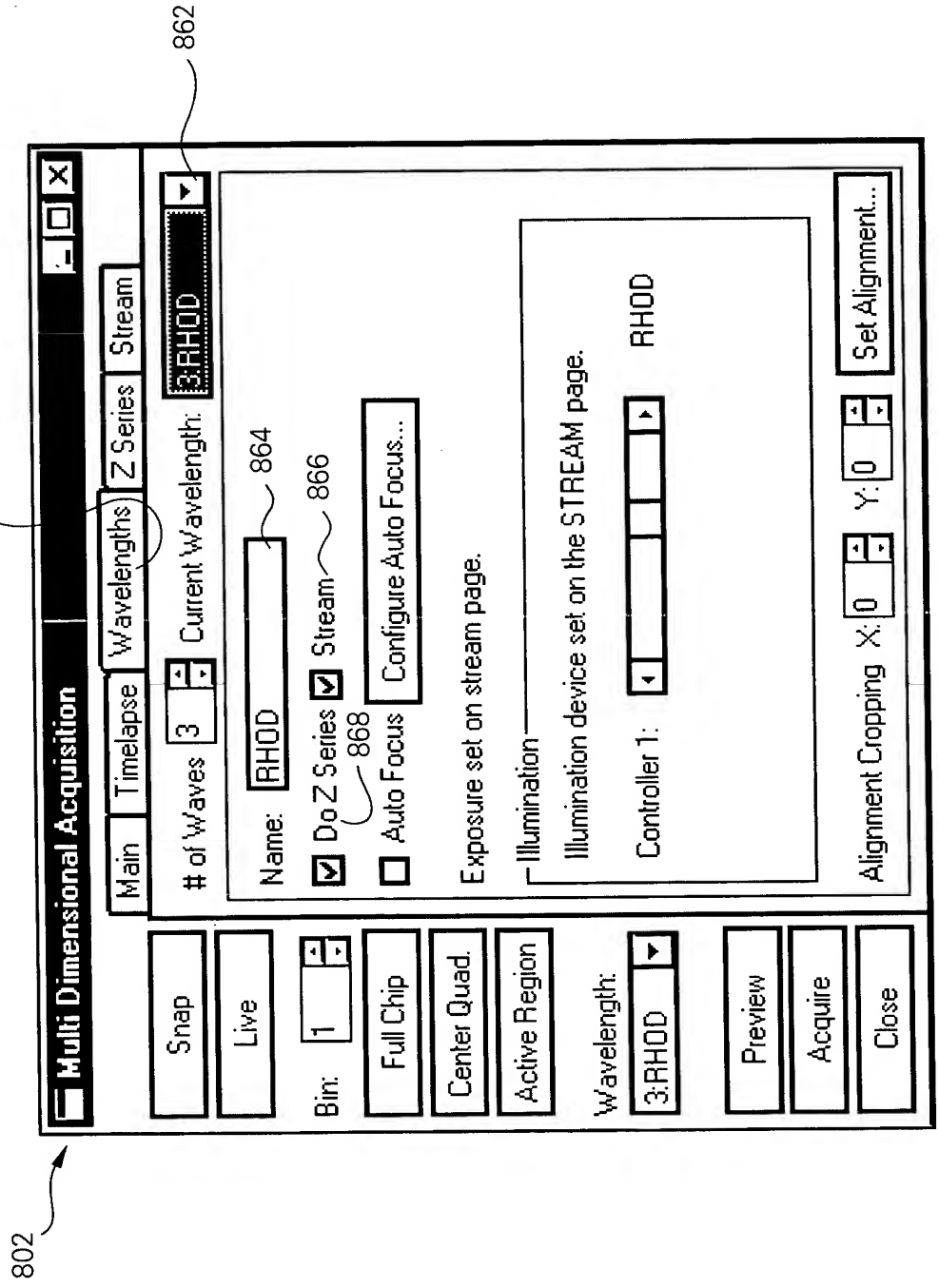
802
808
862
Multi Dimensional Acquisition
Main
Timelapse
Wavelengths
Z Series
Stream
# of Waves
3
Current Wavelength:
3:RHOD
Name:
RHOD
Do Z Series
Stream
Auto Focus
Configure Auto Focus...
Exposure set on stream page.
Illumination
Illumination device set on the STREAM page.
Controller 1:
RHOD
Alignment Cropping X:
0
Y:
0
Set Alignment...
Snap
Live
Bin:
1
Full Chip
Center Quad.
Active Region
Wavelength:
3:RHOD
Preview
Acquire
Close
864
866
868

Fig. 8F

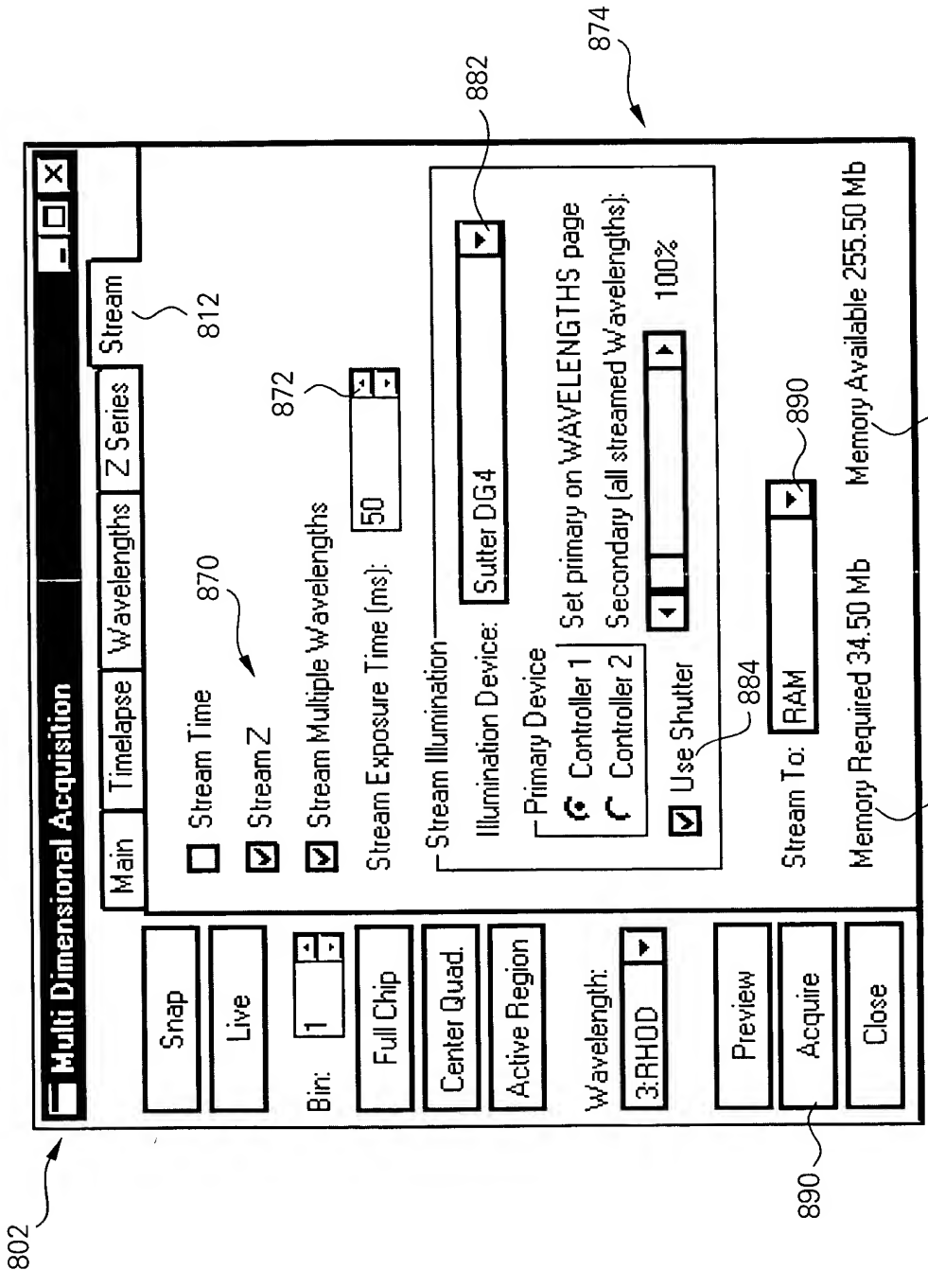
Multi Dimensional Acquisition
Main
Timelapse
Wavelengths
Z Series
Stream
Snap
Live
Bin:
1
Full Chip
Center Quad.
Active Region
Wavelength:
3:RHOD
Preview
Acquire
Close
Stream Time
Stream Z
Stream Multiple Wavelengths
Stream Exposure Time (ms):
50
Stream Illumination
Illumination Device:
Sutter DG4
Primary Device
Controller 1
Controller 2
Use Shutter
Set primary on WAVELENGTHS page
Secondary (all streamed Wavelengths):
100%
Stream To:
RAM
Memory Required 34.50 Mb
Memory Available 255.50 Mb
802
812
870
872
874
878
880
882
884
890
890

Fig. 8G

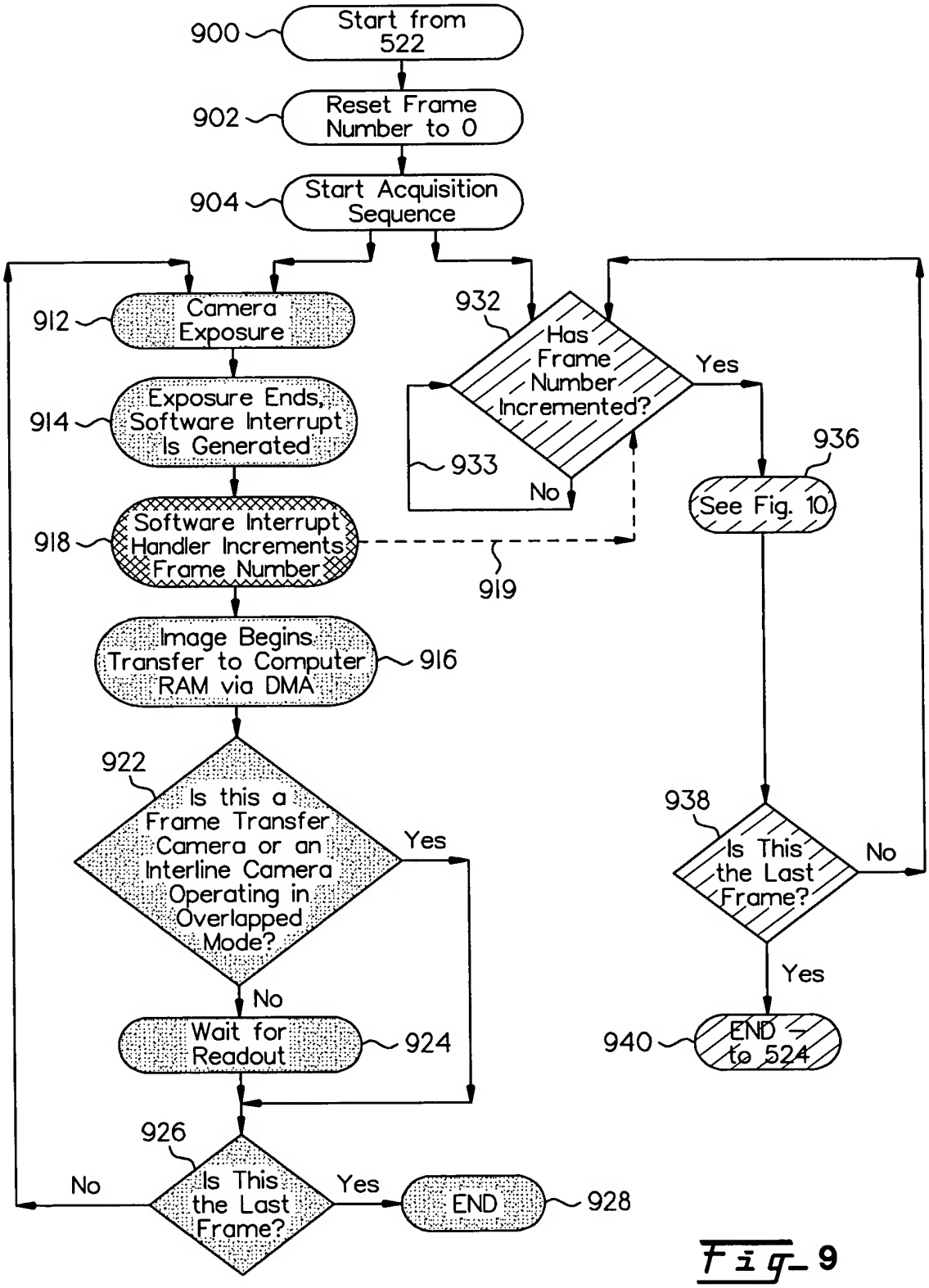
900
Start from 522
902
Reset Frame Number to 0
904
Start Acquisition Sequence
912
Camera Exposure
914
Exposure Ends, Software Interrupt Is Generated
918
Software Interrupt Handler Increments Frame Number
919
Image Begins Transfer to Computer RAM via DMA
916
922
Is this a Frame Transfer Camera or an Interline Camera Operating in Overlapped Mode?
Yes
No
Wait for Readout
924
926
Is This the Last Frame?
No
Yes
END
928
932
Has Frame Number Incremented?
Yes
933
No
936
See Fig. 10
938
Is This the Last Frame?
No
Yes
940
END to 524

Fig. 9

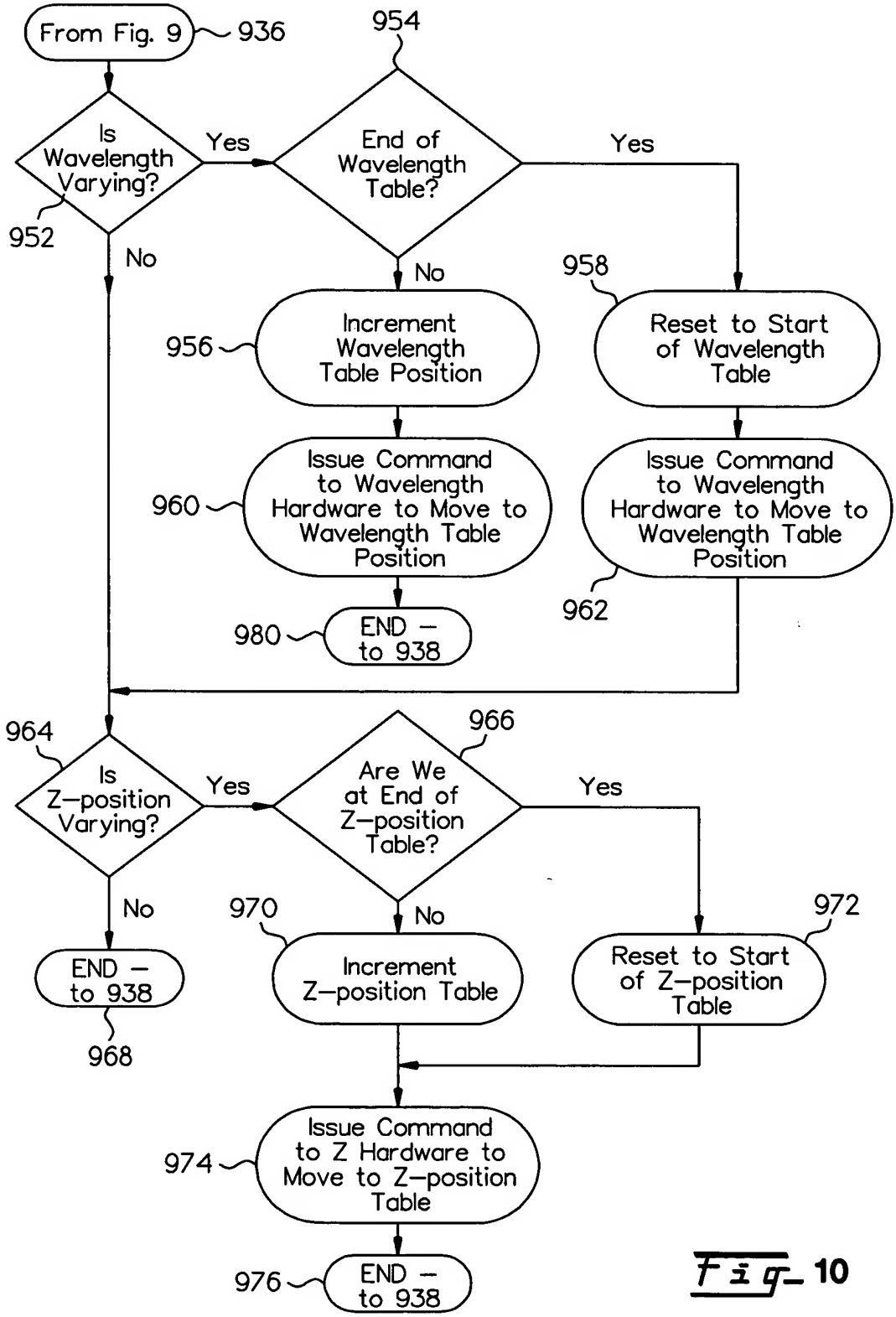
From Fig. 9
936
954
Is Wavelength Varying?
Yes
End of Wavelength Table?
Yes
952
No
No
958
956
Increment Wavelength Table Position
Reset to Start of Wavelength Table
960
Issue Command to Wavelength Hardware to Move to Wavelength Table Position
Issue Command to Wavelength Hardware to Move to Wavelength Table Position
962
980
END – to 938
964
966
Is Z–position Varying?
Yes
Are We at End of Z–position Table?
Yes
No
970
No
972
END – to 938
Increment Z–position Table
Reset to Start of Z–position Table
968
Issue Command to Z Hardware to Move to Z–position Table
974
976
END – to 938

Fig. 10

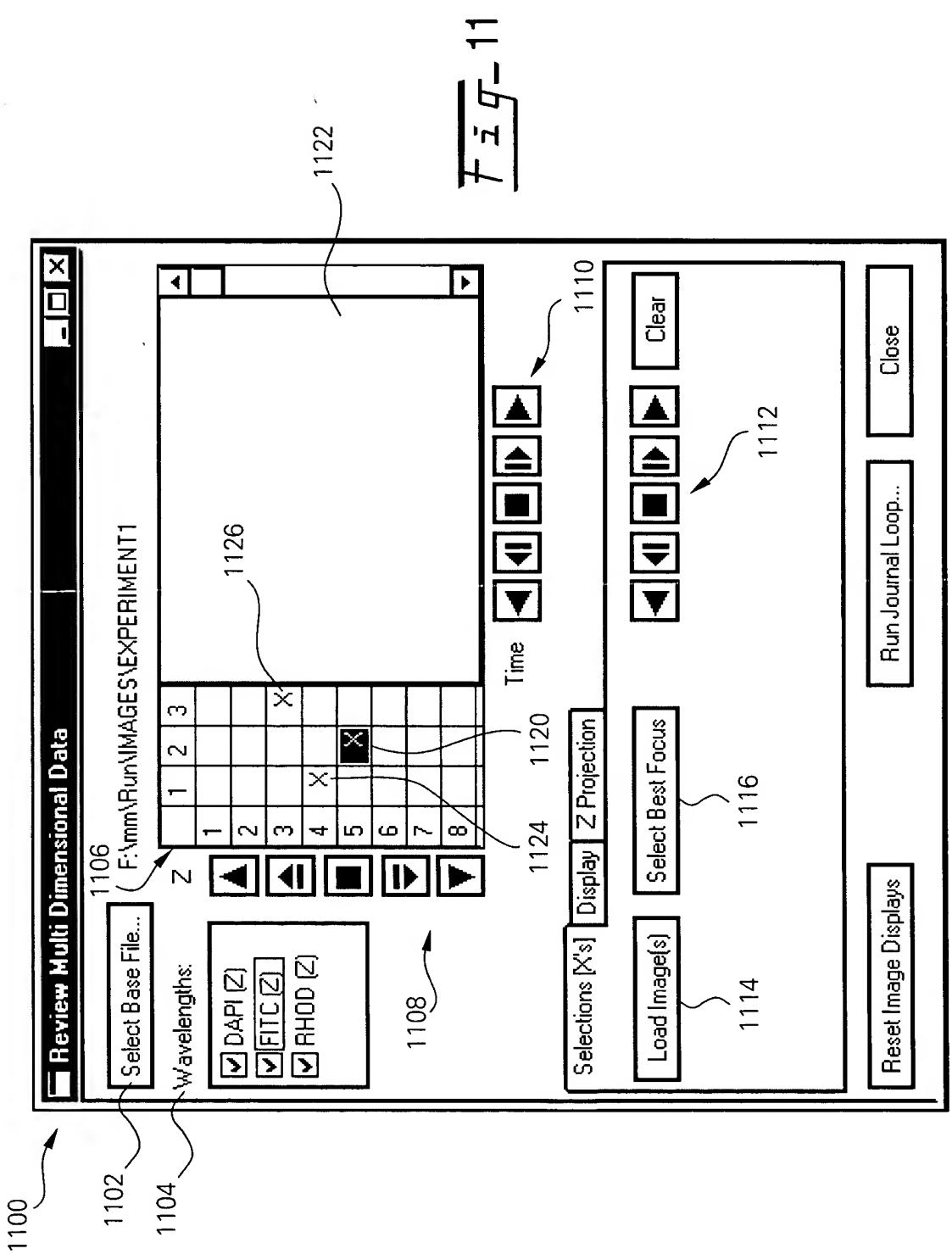
Review Multi Dimensional Data
Select Base File...
F:\mm\Run\IMAGES\EXPERIMENT1
Wavelengths:
DAPI (Z)
FITC (Z)
RHOD (Z)
Z
Time
Selections [X's]
Display
Z Projection
Load Image(s)
Select Best Focus
Clear
Reset Image Displays
Run Journal Loop...
Close
1100
1102
1104
1106
1108
1110
1112
1114
1116
1120
1122
1124
1126

Fig. 11